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Role of endothelium on the abnormal Angiotensin-mediated vascular functions in epileptic rats

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ABSTRACT

Epidemiological studies have found that the risk for cardiovascular disease is increased in patients with epilepsy. The Renin Angiontensin System (RAS), an important player in vascular tone control, is also involved in many neurological disorders, including seizures and epilepsy. Although it has been reported that Angiotensin II (Ang II) release and Angiotensin receptors expression are altered in many cerebral areas in patients/animal models with neurological disorders, there are no data on the vascular function. We evaluated Ang I and Ang II-mediated vascular responses and to correlate their contractile responses to the presence of endothelium and the protein levels of components of the RAS (AT_1, AT_2, Mas and ACE) in aorta isolated from genetically epileptic rats (WAR strain). The major finding was that the vascular contractile response induced by Ang I and Ang II is endothelium-dependent. Ang II induced contractions in aortas from Wistar rats either with intact endothelium (E+) (1.16 ± 0.04 g, n = 6) and endothelium-denuded (E–) (1.24 ± 0.04 g, n = 6). Maximum contractile response (ME) induced by Ang I was lower in Wistar E+ (0.45 ± 0.03 g, n = 6) compared with Wistar E– (1.13 ± 0.08 g, n = 6). Ang I and Ang II failed to induce contraction in WAR E+, whereas the ME induced by Ang I in WAR E– was lower (0.52 ± 0.04 g, n = 11) than in the Wistar. ME induced by Ang II in aortas from WAR was also lower (0.40 ± 0.03 g, n = 11) compared with Wistar. AT1 receptor expression in both E+ WAR and Wistar was lower than in both E– WAR and Wistar. AT_2 and Mas receptor expression was higher in Wistar E– and E+ as compared to WAR E– and E+. ACE expression was higher in both E+ WAR and Wistar, but it was lower in both E– WAR and Wistar. Endothelium impairs the contractile response induced by Angiotensin in WAR, suggesting that endothelial relaxing factors play important role on the aorta contraction.

Keywords: Endothelium; Aorta Myogenic Tone; Angiotensin; Angiotensin II Receptor; Epilepsy

1. INTRODUCTION

Epidemiological studies have found that the risk for cardiovascular disease is increased in persons with epilepsy and it is related to premature death [1-6]. The Renin-Angiotensin System (RAS) is a player in many neurological disorders, including seizures and epilepsy. It has been pointed out that the RAS is a mediator in neurological problems such as the seizures observed in epilepsy and cardiovascular diseases [7].

Considering epilepsy as a neurodegenerative disorder, important studies of Tchekalarova and Georgiev [7] have demonstrated the brain involvement of the RAS in seizure genesis and/or control by activation of the AT_1 receptor, thereby facilitating the discharge firing. Argañaraz *et al.* [8] have reported increased AT_1 receptor expression in the cortex and in the hippocampus of patients with temporal lobe epilepsy, while AT_2 receptor expression was elevated only in the hippocampus of these patients. According to these authors, changes in Angiotensin II (Ang II) release may occur via mechanisms that remain unknown. Ang II would have pro- or anti-epileptogenic function depending on its receptor activation. Their findings suggest that the up-regulation of the AT_1 receptor is related to increased tissue excitability in the hippocampus and cortical areas.

Regarding to the peripheral actions of RAS it is impor-

tant to consider that the RAS plays a relevant role in blood pressure and body fluid homeostasis regulation [9-12]. Angiotensin I (Ang I) is the main substrate for the Angiotensin Converting Enzyme (ACE), yielding the vasoconstrictor Ang II, the main RAS effector [13]. Ang I may also be hydrolyzed by prolyl-endopeptidase and carboxy-peptidases, to yield the vasodilator Angiotensin-1-7 (Ang 1-7) [14]. However, Ang II is the major substrate for (Ang 1-7) synthesis [15]. Besides the AT_1 and AT_2 receptors, the GPCR Mas has been identified as an endogenous receptor for the Angiotensin-(1-7) [16-18].

Ang II exerts its effect through activation of type 1 (AT_1) and type 2 (AT_2) receptors belonging to the family of G-protein coupled receptors (GPCRs). Both types of receptor trigger distinct and sometimes opposing signal transduction pathways [19,20]. The most prevalent physiological and pathological effects of Ang II, such as vascular smooth muscle cell contraction, are mediated by the AT_1 receptor. On the other hand, AT_2 receptors are generally assumed to counteract these actions. Activation of AT_2 receptors leads to several biological processes, such as vascular relaxation [21-24].

It is well known that the RAS and the nitric oxide (NO) system play a crucial part in the regulation of cardiovascular physiology and pathology [25]. According to Yan *et al.* [25] there is a functional relationship between Ang Iotensin II and NO. Nitric Oxide is the main relaxing endothelial factor involved on the aorta relaxation [26,27]. Works using cultured neurons have demonstrated that NO is specifically involved in mediating Ang II actions [28,29]. Ang II is also considered a neurotransmitter/neuromodulator, because it is responsible for the activetion of AT_1 and AT_2 receptors present in several brain regions [30-32]. Although the importance of animal models for the investigation of epilepsy and related disorders is recognized, few studies have been devoted to the relationship between epilepsy and peripheral vascular dysfunctions focusing on endothelium, whose co-morbidities are involved in this neurological disorder.

Growing evidence has indicated the participation of RAS in some neurodegenerative disorders such as Alzheimer's [33,34], Parkinson's [35] and Huntington's [36] diseases. Genetic models of epilepsy have played a crucial role in the elucidation of the pharmacological, electrophysiological, and neuroethological aspects of generalized seizures [37,38]. Of our particular interest is the genetically audiogenic susceptible rodents, the so-called Wistar audiogenic rats (WAR), which present severe co-morbidities [39]. The WAR strain was first obtained by Garcia-Cairasco [40] and has been proven useful for characterization of behavioral sequences in acute audiogenic seizures [41-44], audiogenic kindling studies [45-48], screening of new anticonvulsant agents [49] and research on the neurochemistry of audiogenic seizures [50,51]. Multiple inductions of audiogenic seizures in the WAR strain are a model of temporal lobe epilepsy, due to the recruitment of limbic areas such as hippocampus and amygdale [52].

Although it has been reported that Ang II release and Angiotensin receptors function/expression are altered in many cerebral areas in patients/animal models with neurological disorders, there are no data on the vascular function to Angiotensin peptides of genetically audiogenic susceptible rodents such as the Wistar audiogenic rats (WAR), a model of temporal lobe epilepsy and therefore it has been considered to evaluate the vascular function (vascular reactivity to Angiotensin peptides in WARs.

Therefore, it was hypothesized that the contractile response to Ang I and Ang II is altered in the aorta isolated from rats of the WAR strain. Because of this, our work was designed to evaluate the importance of the endothelium on Angiotensin-mediated vascular responses and to investigate the mechanisms involved in the vascular function of Wistar audiogenic rats.

2. METHODS

2.1. Animals

Male WAR and Wistar rats (Control) aged 80 days were employed. WAR belonged to the inbred strain of the Physiology Department of Faculdade de Medicina de Ribeirão Preto, University of São Paulo, Brazil [23]. Water and food were available *ad libitum* and room temperature was controlled at 23˚C, in a 12 h light/dark circle. All the experiments were performed in accordance with the recommendations of the Brazilian Society for Neuroscience and Behavior for animal experimentation and were approved by the Ethics Commitee on Animal Research of the School of Medicine of Ribeirão Preto, University of São Paulo, Brazil (CETEA, protocol n. 124/2005). All efforts were made in order to avoid any unnecessary animal suffering and to reduce the number of animals employed in the experiments. In order to characterize the model, in its genetic component, it was employed only naïve animals, it was not our aim to obtain epileptic behavior after sound stimulation, *i.e.*, and there were no audiogenic stimuli to induce seizures in the WAR.

2.2. Systolic Blood Pressure Measurement

Systolic Blood Pressure (SBP) was measured in conscious rats by an indirect tail-cuff method (MLT125R Pulse Transducer/Pressure Cuff coupled to the PowerLab 4/S analogue-to-digital converter, AD Instruments Pty Ltd).

2.3. Vascular Reactivity Studies

Rats were killed by decapitation. The thoracic aorta was quickly removed, dissected out, and cut into 4 mm long rings. In some experimental protocols, the effect of

the drugs in endothelium-denuded arteries was studied, in order to avoid interference from endothelium factors. To this end, the endothelium was mechanically removed by gently rolling the lumen of the vessel on a thin wire. The aortic rings were placed between two stainless-steel stirrups and connected to an isometric force transducer (Letica Scientific Instruments; Barcelona-Spain), to measure the tension in the vessels. The rings were placed in a 10 mL organ chamber containing Krebs solution with the following composition (mmol/L): NaCl 130, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 14.9, glucose 5.5, and $CaCl_2$ 1.6. The solution was maintained at pH 7.4 and was gassed with 95% O_2 and 5% CO_2 at 37˚C. The rings were stretched to a basal tension of 1.5 g before allowing them to equilibrate for 60 min in the bath fluid, which was changed every 15 min. Endothelial integrity was qualitatively assessed by the degree of relaxation caused by acetylcholine (1 μmol/L) in the presence of the contractile tone induced by norepinephrine (0.1 μmol/L). For the studies, which required endothelium-intact aortas (E+), the rings that presented more than 90% of relaxation when stimulated with acetylcholine were considered. The arteries were considered endothelium-denuded (E–) if there was not any degree of relaxation upon stimulation with acetylcholine. Then, cumulative effect-concentration curves were constructed for Ang I and Ang II (10 ρmol/L - 10 μmol/L).

2.4. AT_1, AT_2, Mas and ACE Expression

This experimental procedure was designed to quantify the protein expression of AT_1 and AT_2 receptors as well as ACE, as a function of the expression of *β*-actin in control and WAR. Briefly, the aortic rings were isolated from WAR and control. Protein extraction from the tissues with or without endothelium was performed by maceration using lysis buffer consisting of Tris-HCl 10 mmol/L, pH 7.5, NaCl 150 mmol/L, EDTA 1 mmol/L, EGTA 1 mmol/L, SDS 0.1%, Nonidet P-40 1% and the following protease inhibitors: PMSF 2 mmol/L, SBTI 100 mg/mL, leupeptin 10 mg/mL, aprotinin100 mg/mL, benzamidin 10 mmol/L, and sodium orthovanadate 2 mmol/L. After 30 min of homogenization at 4˚C, the lysate was centrifuged for 15 min at 4˚C and 13,000 rpm, and total protein was measured using a commercial kit (Anresco). Then, 5 - 20 mg of total protein was separated by SDS-PAGE (12% for the Ang II receptors and 10% for ACE) and transferred to a nitrocellulose membrane, and Western Blotting was performed for AT_1, AT_2, and Mas receptors and for ACE. The membrane blocked with 3% BSA (in TTBS). The primary antibodies were diluted in TTBS + 3% BSA, 1:1000. The secondary antibodies were diluted in 1:3000 (anti-rabbit, purchased from Santa Cruz). The bands were developed by using an enhanced chemiluminescence kit. The membranes were deblotted, and Western Blotting for *β*-actin was developed.

2.5. Drugs

Acetylcholine, norepinephrine, Angiotensin I, and angiotensin II were obtained from Sigma Chem. Co. (St Louis, MO-USA). Solutions of the drugs were prepared in deionized water. All the antibodies employed in the Western Blotting analyses were purchased from Santa Cruz Biotechnology, Inc.

2.6. Data Analysis

Data are expressed as mean ± SEM. In each set of experiments, *n* indicates the number of studied rats.

The Western Blotting data were expressed by the intensity of the bands, which were measured by densitometry. Densitometric values obtained with the aid of the Image J program (http://rsb.info.nhi.gov/ij/) were used to calculate the rate of AT_1 and AT2 receptors and ACE as a function of the *β*-actin expression, and the corresponding results were plotted using the Graph-Pad software (GraphPad, San Diego, CA).

The data were statistically analyzed by Student's t-test or by ANOVA (one-way), followed by the Newman-Keuls post-test. Tests were employed according to the data. A value of $p < 0.05$ was considered significantly different.

3. RESULTS

The systolic pressure measured by the tail-cuff method was higher in WAR (120 ± 2 mmHg, n = 15, $p < 0.05$) as compared to the Wistar (90 ± 2.0 mmHg, n = 16) (Figure not shown).

In order to study the vascular reactivity to the agonists of the renin-Angiotensin system (RAS), it was constructed concentration-effect curves for Ang I and Ang II. The influence of endothelium on the contractile responses induced by Ang I and Ang II in aortic rings isolated from WAR and Wistar was examined by using intact-endothelium (E+, **Figures 1(a)** and **1(b)**) and denuded (E–, **Figures 2(a)** and **(b)**) arteries.

As shown in **Figure 1(a)**, in Wistar E+ both Ang I and Ang II induced contractile response with the maximum effect (ME) of 0.45 ± 0.03 g (n = 6) and 1.16 ± 0.04 g (n = 6), respectively. This response was significantly higher for Ang II than Ang I ($p < 0.01$). On the other hand, Ang I (ME: 0.10 ± 0.008 g, n = 10) and Ang II-induced contractile responses (ME: 0.09 ± 0.008 g, n = 7) were almost abolished in aortic rings from WAR E+ (**Figure 1(b)**).

In order to investigate if this response was abolished only to RAS agonists, the contractile response to phenylephrine as an adrenergic agonist was also evaluated in intact endothelium aortic rings. Differently of the Ang I and Ang II-induced responses, PHE was able to trigger contractile response in both Wistar (ME: 2.0 ± 0.06 g, n = 6) (**Figure 1(a)**) and WAR (ME: 1.50 ± 0.02 g, n = 9) (**Figure 1(b)**).

As depicted in **Figure 2**, it was possible to obtain contractile response for both Ang I and Ang II in denuded aortic rings. However, the contractile responses induced by Ang I (ME: 0.52 ± 0.04 g, n = 11, $p < 0.001$) and Ang II (ME: 0.40 ± 0.03 g, n = 11, $p < 0.05$) were lower in WAR (**Figure 2(b)**) than the response observed in the Wistar (**Figure 2(a)**) for Ang I (ME: 1.13 ± 0.08 g, n = 11) and Ang II (ME: 1.24 ± 0.04 g, n = 11).

It was evaluated whether this differential vascular reactivity was due to the different expression of the RAS components in the aortic tissue with intact endothelium and in endothelium-denuded aortic rings isolated from WAR and Wistar rats. As illustrated in **Figure 3**, the AT_1 receptor protein expression was increased upon endothelium removal in both WAR and Wistar. Thus, AT_1 expression in WAR E+ (1.21 ± 0.09, n = 4) and Wistar E+ (1.16 ± 0.05, n = 4) were lower ($p < 0.05$) compared with WAR E– (1.60 ± 0.14, n = 4) and Wistar E– (1.50 ± 0.09, n = 4), respectively. There were no statistical differences

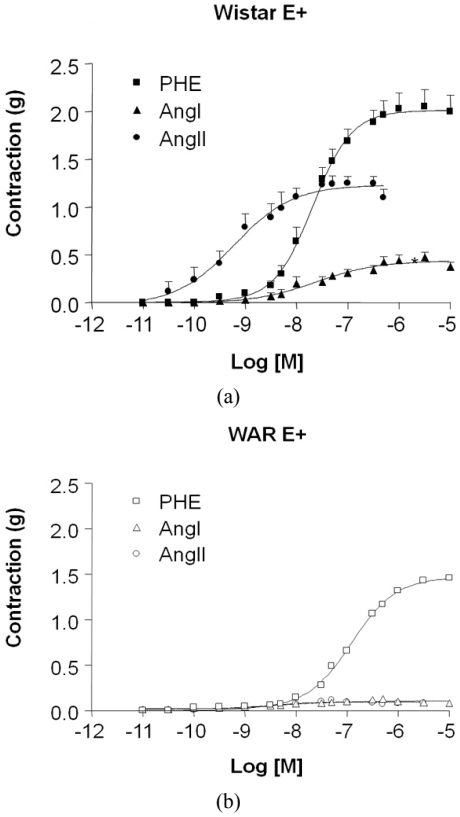

Figure 1. Contractile response induced by angiotensin I (Ang I), Angiotensin II (Ang II) and phenylephrine (PHE) in intact endothelium (E+) aortic rings isolated from Wistar (a) and WAR (b) rats. Cumulative concentration-effect curves for Ang I, Ang II and PHE were constructed in E+ aortic rings isolated from Wistar (control, n = 6) and WAR (n = 10) rats. Results are presented as mean ± SEM and are expressed as grams (*g*) of tension.

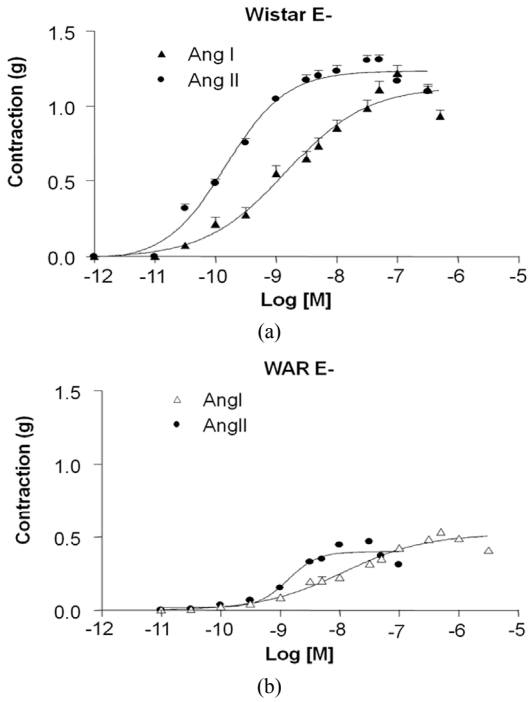

Figure 2. Contractile response induced by Angiotensin I (Ang I) and Angiotensin II (Ang II) in denuded endothelium (E–) aortic rings isolated from Wistar (A) and WAR (B) A: Cumulative concentration-effect curves for Ang I and Ang II were constructed in E– aortic rings isolated from Wistar rats (control, n = 11) and WAR rats (n = 11). Results are presented as mean ± SEM and are expressed as grams (*g*) of tension.

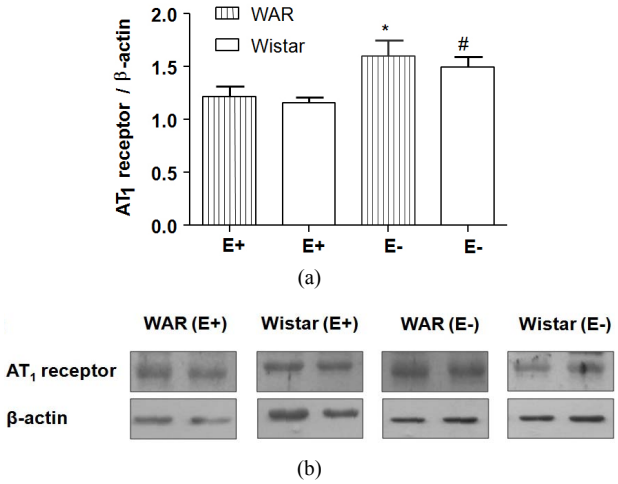

Figure 3. AT_1 receptor protein expression in intact endothelium (E+) and denuded (E–) aorta from WAR and Wistar rats. Representative image of Western Blotting analysis (a) and densitometric group data (b) evaluating protein expression of the AT_1 receptor in aortas from WAR and Wistar rats. Results are presented as mean ± SEM (n = 4). *Indicates difference between the group WAR E– compared with WAR E+ ($p < 0.05$). #Indicates difference between the group Wistar E– compared with Wistar E+ ($p < 0.05$). Data were analyzed by using one-way ANOVA (post test: Newman-Keuls multiple comparisons test).

between WAR E– and Wistar E– or WAR E+ and Wistar E+.

According to the Western Blotting analysis, the AT_2 receptor expression was lower in both WAR E– (0.51 ± 0.06, n = 4, $p < 0.05$) and WAR E+ (0.52 ± 0.07, n = 4, $p < 0.001$) than in Wistar E– (1.30 ± 0.5, n = 4) and Wistar E+ (2.10 ± 0.4, n = 4), respectively (**Figure 4**). The presence or the absence of endothelium did not alter the expression of AT_2 receptor either WAR or Wistar rats.

As represented in **Figure 5** the expression of the Mas receptor in WAR E+ (0.13 ± 0.02 n = 4) and WAR E– (0.16 ± 0.06, n = 4) was lower than its expression in Wistar E+ (0.39 ± 0.07, n = 4, $p < 0.05$) and Wistar E– (0.54 ± 0.02, n = 4, $p < 0.001$), respectively. The presence or the absence of endothelium did not alter the expression of Mas receptor either WAR or Wistar rats.

As shown in **Figure 6**, ACE expression was higher in both WAR E+ (1.41 ± 0.4, n = 4, $p < 0.001$) and Wistar E+ (1.15 ± 0.05, n = 4, $p < 0.001$) than in denuded aortas from WAR and Wistar, respectively. Endothelium removal decreased ACE expression in both WAR (0.28 ± 0.06, n = 4) and Wistar (0.11 ± 0.03, n = 4).

4. DISCUSSION

This is the first study concerning the involvement of the RAS on the peripheral vascular activity in an animal model to study epilepsy. It was evaluated the implications of the agonist of the RAS in the contractile dysfunction on the animal model of epilepsy, the WAR strain.

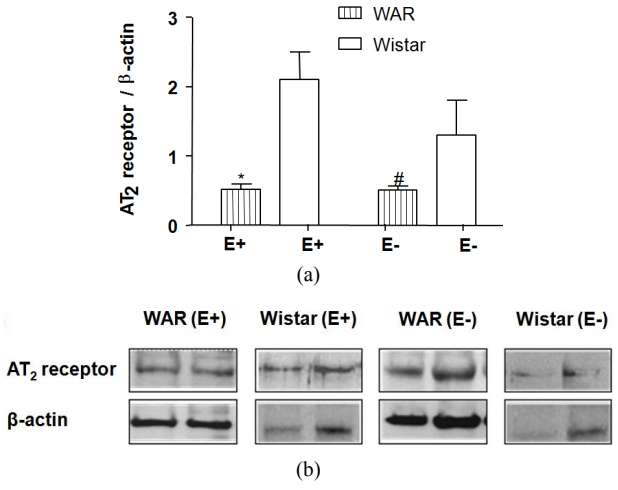

Figure 4. AT_2 receptor protein expression in intact endothelium (E+) and denuded aorta (E–) from WAR and Wistar rats. Representative image of Western Blotting analysis (a) and densitometric group data (b) evaluating protein expression of the AT_2 receptor in aortas from WAR and Wistar (control) rats. Results are presented as mean ± SEM (n = 4). *Indicates difference between E+ WAR and E+ Wistar ($p < 0.001$). #Indicates difference between E– WAR and E– Wistar ($p < 0.05$). Data were analyzed by using one-way ANOVA (post test: Newman-Keuls multiple comparisons test).

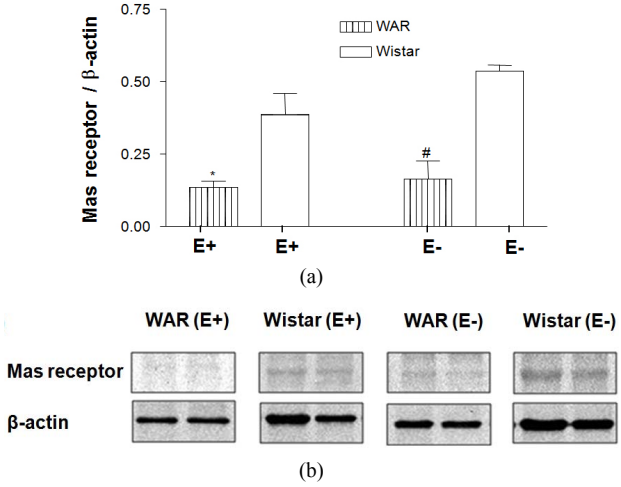

Figure 5. Mas receptor protein expression in intact endothelium (E+) and denuded (E–) aorta from WAR and Wistar rats. Representative image of Western Blotting analysis (a) and densitometric group data (b) evaluating protein expression of the Mas receptor in aortas from WAR and Wistar E+ and E–. Results are presented as mean ± SEM (n = 4). *Indicates difference between E+ WAR and E+ Wistar ($p < 0.05$). #Indicates difference between E– WAR and E– Wistar ($p < 0.001$). Data were analyzed by using one-way ANOVA (post test: Newman-Keuls multiple comparisons test).

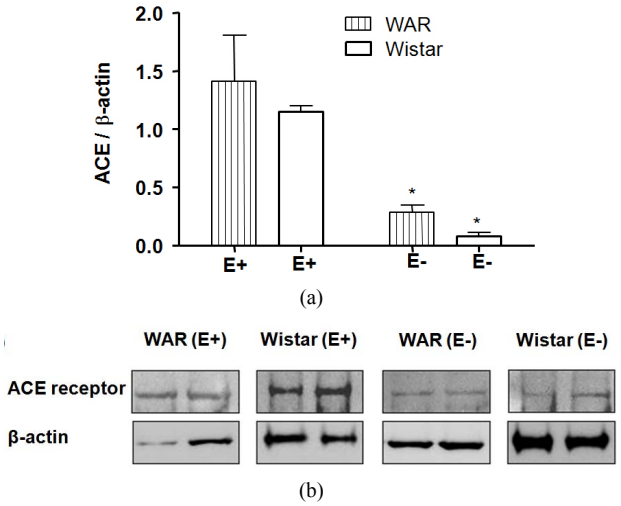

Figure 6. Angiotensin-Converting Enzyme (ACE) protein expression in intact endothelium (E+) and denuded (E–) aorta from WAR and Wistar rats. Representative image of Western Blotting analysis (a) and densitometric group data (b) evaluating protein expression of ACE in aortas from WAR and Wistar E+ and E–. Results are presented as mean ± SEM (n = 4). *Indicates difference between the WAR/Wistar E– group compared to the WAR/Wistar E+ group ($p < 0.001$), respectively. Data were analyzed by using one-way ANOVA (post test: Newman-Keuls multiple comparisons test).

It is well known that central and peripheral structures are affected by RAS activity in a way that one response influences others and vice-versa. Considering the epilepsy

disorder, this relationship could contribute to the co-morbidities, including cardiovascular alterations. Although Ang II has been assumed to be the main peptide acting in the periphery, a large number of studies support the concept that it acts as a neurotransmitter or neuromodulator in specific neuronal pathways in the central nervous system [53-56]. In the present study, our experimental approach was focused on the peripheral vascular function of WAR a genetically developed strain [39], in animals who had not been stimulated for developing audiogenic seizures. Although it is not classified as a hypertensive status, in this study it was demonstrated that the level of systolic blood pressure of WAR was higher than that of the Wistar control group. This increased blood pressure level may be of concern because the impaired cardiovascular system is one of the possible mechanisms of cause or effect of the development of such seizures and the prevalence of sudden unexplained death in epileptic patients-SUDEP [57,58]. According to Scorza *et al.* [59], the abnormalities in cardiovascular system are involved in SUDEP. Since hypertension is an important risk factor for cardiovascular diseases, the increasing systolic blood pressure in epileptics could represent a point of the surveillance in the clinical practice. Adaptive cardiovascular reflexes could explain increased arterial pressure in WAR during seizures contributing to the cardiac manifestations that are integrated with the sympathetic and/or parasympathetic discharges triggered by seizures [60].

Our results show that the Ang II-induced vasoconstriction was potentiated by endothelium removal. These data are in accordance to Boulanger *et al.* [61]; Caputo *et al.* [62]; Gruetter *et al.* [63]; and Zhang *et al.* [64], who have demonstrated that this vasoconstriction is decreased by the release of endothelium-derived relaxing factors such as NO in non-epileptic vessels. In the present study, endothelium impaired the contractile response induced by both Ang I and Ang II. It has been reported that NO and Ang II have opposite effects on vascular responses as blood pressure regulators [65]. Since endothelium reduced the contractile response to Ang I and Ang II in aorta isolated from control (non-epileptic) rats, we suggest that an endothelium-relaxing factor could be involved in the lower response to these peptides. Otherwise, in Wistar rats relaxing endothelium-derived factors do not influence the contractile response induced by Ang II, since its response was similar in the presence and absence of endothelium. Other studies have also shown the dependence on endothelium of the contractile response induced by both Ang I and Ang II [66]. In addition, our results suggest that the RAS system rather than the adrenergic response is affected by endothelium in aorta from epileptic rats.

In the present study, it is interesting to note that the expression of AT_1 receptors in E+/E– WAR aorta was similar to their expression in E+/ E– Wistar aorta, despite the differences in the contractile responses to Ang I/Ang II observed in E– WAR and in the E+ WAR aortas. Considering the AT_1 receptor expression, one can suggest that it contributes to the higher contractile response in E– WAR as compared to the one detected in E+ WAR.

Due to vascular responses, AT_1 receptor activation and AT_2 receptor activation have opposite effects on blood pressure [65]. The activation of AT_2 receptors by Ang II induces endothelium-dependent vasodilation in vascular beds [66]. In this sense, we have evaluated the AT_2 receptor expression. As reported by Pueyo & Michel [67] and Harbel *et al.* [68], the Ang II receptors are mainly of the AT_1 type in rat endothelial cells, but AT_2 receptors have also been found. Herein, AT_2 receptor expression in WAR aorta was not altered by the presence or absence of endothelium. AT_2 receptor expression was higher in the Wistar animals compared with WAR, regardless of E^+ or E– aortas. Otherwise, a tendency to lower AT_2 expression was observed for Wistar E– aorta, which could explain the lower amplitude of the contractile response induced by Ang I and Ang II.

Although AT_1 receptor activation and AT_2 receptor activation have opposite effects, it has not yet been confirmed that the AT_1/AT_2 receptor heterodimer inhibits AT_1 signals [67]. Therefore, Mas receptor investigation could be helpful in explaining our results about the absence of the contractile response to Ang I and Ang II stimulation in the presence of endothelium since Ang (1-7) acts as an endogenous antagonist of Ang I/Ang II and blocks Ang II-mediated vasoconstriction and cell proliferation [69,70]. In our hands, Mas expression was higher in control Wistar compared with WAR aorta, in an endothelium-independent way. These results support the statement that the Mas receptor is not responsible for the differences observed in the vascular reactivity responses in WAR.

In the present investigation, ACE expression was higher in E+ WAR and E+ Wistar aorta compared with E– WAR and E– Wistar. These results suggest that a higher level of Ang I could be converted to Ang II in WAR.

The removal of the endothelium suppresses relaxing regulator factors, mainly NO. To some extent, Ang II acting on smooth muscle cells triggers a contraction that is not counterbalanced by its vasodilatory effects endothelium-dependent. It seems that in WAR animal, endothelium exerts a vasodilatory control stronger than that existing in control Wistar rats. In fact, Ang II contractile response is modified in WAR rat and the regulation by endothelium is in part involved in that alteration of Ang II response.

5. CONCLUSION

The presence of endothelium impairs the contractile

response induced by both peptides Ang I and Ang II in WAR aortas. It suggests that endothelial factors could have an important role in protecting the aorta vascular smooth muscle contraction stimulated by these peptides in this genetically epileptic rat strain, whose systolic blood pressure is lightly increased in naïve conditions (without developing seizures).

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